Fabrication and Characterization of Camphor based Nanoformulation and Bioefficacy against *Pediculus humanus capitis*

SYNOPSIS

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INTRODUCTION

Parasites are the major problem worldwide. Both ectoparasites and endoparasites cause various types of infections in humans as well as in animals. Head lice infestation and nits are the major problem in humans as they cause too much of itching. Lice are small wingless insects that live as ectoparasites of mammals and birds. They are usually classified within the order phthiraptera, although some authors have placed them in two separate orders, the mallophaga, which includes the chewing lice, and anoplura, the sucking lice. *Pediculus humanus capitis* is a holometabolous insect, completing its entire cycle on head hair, from eggs (nits) to adults through three nymph instars, in about 45 days (Takano-Lee *et al*., 2003).

The most common species of head lice, *Pediculus humanus capitis* is found in humans mostly in school going children (Mumcuoglu *et al.,* 2009). Other species of lice are found in animals and birds, which are throbbing to them. Moreover, girls are more and very frequently infested as compared to boys (Downs *et al.,* 2000; Gallardo *et al.,* 2012). Head lice can be transmitted to one another by direct contact during playing, sleeping with the infested person etc. Yet, there are no direct evidence that head lice are acting as a vector but, few experimental tests have shown that they can act as vectors of *Rickettsia prowazekii* and *Bartonella quintana*, the causative agents of typhus and trench fever respectively (Robinson *et al.,* 2003; Sasaki *et al.,* 2006; Gallardo *et al.,* 2012). According to the report in 2008, overall infestation prevalence rate of head lice was 81.5%, the highest values corresponding to children from 6 to 11 years of age, with a slight predominance in males approximately 55.4% *vs.* 44.6% (Chouela *et al.,* 1997). Presence of lice in the head shows unhealthy status and it causes itching hence, it should be controlled by applying oils and shampoos.

The potential world market for products that control pediculosis is quite large. Extensive lists of available products that have been approved for head lice treatment may be found elsewhere (Jones and English, 2003; Burgess *et al.,* 2004; Burkhart and Burkhart, 2006). Chemical based shampoos and oil causes irritation and has adverse effect on eyes too. They also develop resistance to the target organism therefore herbal products could be used or apply for the control of *Pediculus humanus capitis*.

The need for alternative options for head lice treatment, combined with the growing tendency to use non- toxic, "natural" products, particularly since children are the usual recipients, has prompted a number of scientists to search for new control products from plants, products that should combine good anti-lice activity, low levels of resistance and better safety (Ghaffar *et al.,* 2010). Medicinal plant based products are used for a long time to treat the head lice problem. Neem (*Azadirachta indica*) has the widest spectrum of use of all natural products. The first known use of neem by the Harappa culture in ancient India dates back 4,500 years (Dasgupta *et al*., 2004) for head lice. Neem also shows very good anti- bacterial, antifungal and larvicidal properties also few reports are available on pediculicidal activity of neem (Heukelbach *et al.,* 2007; Ghaffar *et al.,* 2012). Products based on cinnamon, marjoram, pennyroyal, rosemary, *Lippia multiflora*, and *Eucalyptus* oils were effective against head lice in vitro (Oladimeji *et al*., 2000; Yang *et al*., 2004; Yang *et al*., 2005). Camphor (*Cinamomum camphora*) has many properties, it was used from traditions with carrier oil for the treatment of dandruff and head lice as it has potential to unconscious the lice. Lemon (*Citrus limon*) juice also possesses pediculicidal activity (Shrivastava *et al.,* 2010). Moreover, ginger (*Zingiber officinale*) used in many countries as a spice and condiment to add flavor to food. (Park *et al.,* 2002) Besides this, the rhizome of ginger has also been used in traditional herbal medicine and the health-promoting perspective of ginger is attributed to its rich phytochemistry.

Since last decade, nanoformulations are being widely used for controlling various insects like ticks, mosquitoes and other pests etc. (Nenaah *et al.,* 2015; Galli *et al.,* 2018). Here, using nanotechnology we propose to enhance bioactivity of certain botanicals with promising activity through reduction in the particle size. By using nanotechnology, we will be able to prepare green nanoformulation with small droplet size and better penetration and enhanced activity for controlling head lice which is persistent problem in children and no attention has been paid to control this which is evidenced by scanty studies in India in spite of being second most populous nation of the world. It may be linked with the poor performance of children in the school as there is a report that parasitic infection affects the academic performance of children (Nokes *et al.,* 1992).

Therefore, the present study is planned to test pediculocidal activity of neem, lemon and ginger oils with camphor nanoformulations against adults and late nymph of *Pediculus humanus capitis.*

REVIEW OF LITERATURE

There are more than 3000 species of lice present in the world. Among these, humans constitute the preferred host only for the two species namely, *Pediculus humanus* (head lice) and *Phthirus pubis* (pubic lice). It sucks the blood and causes irritation, itching on the scalp and redness. Chemical based shampoos and products are not of much interest owing to their harmful impacts and resistance development (Brandenburg *et al*., 1986). Now a day's rather oils and plant based products are in the trend, so here we have reported a review and further planned the research work.

Morsy *et al*. (2000) reported that neem extract had killed head lice slowly *in vitro* in a filter paper test and was less effective than malathion, permethrin and benzyl benzoate. However, the units of concentration of active ingredient reported in the paper are confusing. In the same study, lemon (*Citrus limon*) extract had shown less effect on head lice than neem extract, and aloe vera extract had no killing effect after 3 hours. Tiangda *et al.* (2000) prepared custard apple seed extract which was found tobe highly effective against head lice in festations in a small comparative trial. More than 90% of lice were dead three hours afterapplication of the product to infested school children, as compared to 60 % dead lice in the control group, treated with 25% benzyl benzoate emulsion. Downs *et al*. (2000) conducted an *in vitro* study against head lice showed that 1% tea tree oil caused no mortality after 2 hours while 10% extract resulted in 86%mortality. Takano-Lee *et al.* (2004) studied the home remedies to control head lice by assessment of homeremedies to control the human head lice. Yang *et al.* (2004) reported ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis*. Heukelbach *et al.* (2006) formulated a new shampoo based on neem which is highly effective against head lice. Rossini *et al.*(2008) studied the plant extracts and their components as potential control agents against human headlice.

Khater *et al.* (2009) studied on lousicidal, ovicidal and repellent efficacy of some essential oils against lice and flies infesting water buffaloes in Egypt. The lousicidal and repellent efficacy of five essential oils were investigated for the first time against buffalo louse. Lemon has been used for

both culinary and medicinal purposes. Shrivastava *et al.* (2010) studied in vitro pediculicidal activity of juice of *Citrus limon*. Schmahl *et al.* (2010) studied the efficacy of neem seed extracts on a broad spectrum of pests and parasites. Some target species are less sensible beetles, tristoma stage, fly maggots while the specimens ofthe other species cited above were successfully killed even at low concentration of the extract. Ghaffar *et al*. (2010) reported repellency against head lice *(Pediculus humanus capitis).* This study showed that a combination of seed extract of the plant *Vitex agnus castus* (monk pepper) and the compound paramenthan-3,8-diol (which is also found in some plants, e.g. *Eucalyptus*) act synergistically and are able to protect human hair for at least 7 h from invasion of head lice (*Pediculus humanus capitis*). The recently developed product containing both compounds is named Licatack Preventive Audino *et al.* (2011) studied comparative toxicity of oxygenated monoterpenoids in experimental hydroalcoholic lotions to permethrin-resistant adult head lice. Ghaffar *et al.* (2010) performed a clinical trial on the efficacy of grapefruit extract on head lice. Jayaseelan *et al.* (2011) synthesized silver nanoparticles by leaf extract from heartleaf moonseed plant against *Tinospora cordifolia* (Miers). Mehlhorn *et al.* (2011) studied the ovicidal effects of a neem extract preparation on eggs of body and head lice. The eggs of head lice and body lice were incubated for 5, 10, 15, 20, 30 or 45 min in to neem seed extract contained in a fine shampoo formulation, which is known for its significant killing effects of larvae and adults of head lice. Ghafar *et al*. (2012) studied the efficacy of neem seed extract against head lice for nits and motile stages. Brown and Burgess (2017) performed a study to find neem oil as an effective hair lotion on the head lice. The product consisted of a cetearyl alcohol, glyseryl mono stearate, and liquid paraffin conditioner base with the addition of 1% neem seed oil, 0.25% tea tree oil, 0.25% lavender oil and 1%nettleextract. Lucia *et al.* (2017) prepared novel polymeric micelles for insect pest control by encapsulating essential oilmonoterpenes inside a triblock copolymer shell for head lice control. Santos *et al.* (2019) studied the evolution of hair treatment and care by the prospects of nanotube-based formulations.

Therefore, the present study will be focusing on enhancing the pediculicidal activity of camphor based neem, lemon and ginger oil nanoemulsions.

Objectives

The following objectives will be undertaken for the study:

- Preparation and characterization of camphor based nanoformulations of ginger, lemon and neem.
- Evaluation of pediculicidal activity of prepared nanoformulations against *Pediculus humanuscapitis*.

MATERIALS AND METHOD

MATERIALS:

1. **Experimental animal-*Pediculus humanus capitis***

Taxonomic classification:

Domain	Eukaryota
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Phthiraptera
Family	Pediculidae
Genus	*Pediculus*
Species	*humanus*

Figure 1 ***Pediculus humanus capitis***

Geographic distribution: Head lice occurs more in the hair of females than male because females have longer hair than male. Head lice are dangerous infection spread across the world.

Medical importance: The main stream of head lice infestations is a symptomatic. Symptoms are tickling feeling in the hair, sores from scratching irritability and difficulty sleeping, frequent itching etc. Head lice are most active in the dark. Body lice can serve as vectors for *Rickettsia prowazekii* (epidemic typhus), *Bartonella quintana* (trench fever), and *Borrelia recurrentis* (louse-borne relapsing fever).

Modes of Transmission: Head lice are extremely contagious. Close contact or sharing personal belongings, such as hats or hair brushes, puts people at risk. Children ages 3-11 and their families get head lice most often personal hygiene has nothing to do with getting head lice.

Life cycle: There are three stages present in the life cycle of head lice: nits, nymph, and adult.

Nits: Head lice eggs are known as nits. Female head lice lay 7-8 eggs in 1 day. They are 0.8 mm by 0.3 mm, oval and usually yellow to white. Nits take 1 week to hatch.

Nymphs: The egg hatches in nymph. The nit shell then becomes a more visible dull yellow and remains attached to the hair shaft. The nymph looks like an adult head louse, but is small in size as compared to adult.

Adults: Nymphs slowly turn into adult. 3 pair legs are found in the head lice and six in number. Females are usually larger than male. Female head lice lay eggs above 7 in 1 day.

2. Cinanamomum camphora

Taxonomic classification:

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Laurales
Family	Lauraceae
Genus	*Cinnamomum*
Species	*camphora*

Figure 2 *Cinanamomum camphora*

Camphor is well known as a natural remedy, and comes from

the distilled wood of the camphor tree, will be used to synthesize nanoformulation against head lice. Camphor is mostly used in the form of inhalants and of camphorated oil, a preparation of 20% camphor incarrier oil, for the home treatment of cold and as a major active ingredient of ointments and balm used as tropical analgesics. This oil is often used as are pellent and is a common ingredient in moth balls. It is not recommended to take this internally as it can have drug like effects.

3. Azadirachta indica

Taxonomic classification:

Kingdom	Plantae
Division	Tracheophytes
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	*Azadirachta*
Species	*indica*

Figure 3 *Azadirachta indica*

Neem oil is one of the best natural remedy, used since a long period of time for head lice. It is said that neem oil not only kill off the lice, but also helps with improving many of the side effects associated witha lice infestation, especially itchiness and irritation of the scalp. In our study we will be using cold pressed oil of neem. It has native the Indian subcontinent, and has been used as a natural medicine in India since ancient times.

4. Citrus limon

Taxonomic classification:

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Rutaceae
Genus	*Citrus*
Species	*limon*

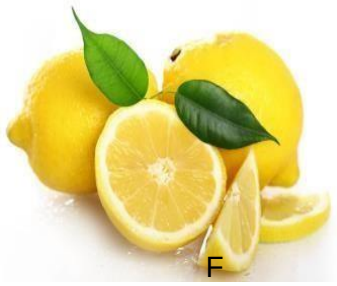

Figure 4 *Citrus limon*

Lemon essential oil is cold pressed from the outer peel of the lemon fruit. Due to its wonderful fragrance, lemon oil is often used in aromatherapy. A few drops can be placed in an oil burner or diffuser to calm anxiety. We can add a few drops to our regular shampoo to destroy fungus which may be causing a flaky scalp. This is a fast and effective remedy, especially when combined with

coconut oil. It is commonly used to clean grease, oil and dirt from guitar fret boards. Be sure to purchase lemon oil which is marketed for guitars. We can add 20 drop of lemon oil to a little vinegar to create a powerful and natural cleaning agent. Purchase some natural soap bars and melt them in a heavy bottom saucepan. add a few drops of lemon oil, or make combination with our favourite essential oils.

5. Zingiber officinale

Taxonomic classification:

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Zingiberales
Family	Zingiberaceae
Genus	*Zingiber Mill.*
Species	*officinale*

Figure 5 *Zingiber officinale*

Ginger has been used for thousands of years-not only in the far east, but also in Europe. Different culturesincorporate this spicy root into their dishes because it is so flavourful, but also because of its health benefits. in India they grate it into their tea, while in Japan ginger is used in miso soup, and in Greece it goes well with fish. However, there's another great way to enjoy ginger's benefit such as it has anti- inflammatory, antibacterial, antiviral and other healthful properties. Ginger is also used as a medicine.

METHODOLOGY

Procurement of botanical oils and chemicals: All the selected oils would be procured from authorized dealer with certificate of analysis and will be further subjected to GC-MS analysis for active component evaluation. Camphor will be obtained from the local market while surfactant (Tween 80) would be purchased from Merck.

Preparation of nanoformulation: Different ratios of oil in water nanoemulsion will be formulated using sonication which will result in the particle size to be smaller using the method of Sugumar *et*

al. (2014). Nanoemulsions will be prepared by the spontaneous emulsification method. They can be prepared simply by oil, water, surfactant, and camphor in the magnetic stirrer, with sonication using the protocols already optimized in the laboratory. Initially, single oil nanoemulsion will be prepared with camphor with varying oil: surfactant ratios (1: 0.5, 1:1, 1:1.5, 1:2). Thereafter, combination of oils would be used along with camphor to study the synergism in bioactivity.

Characterization of nanoformulation: For characterization, initially thermal stability of prepared formulations will be analyzed on different temperatures for about three months thereafter, the most stable ratio will be subjected to Dynamic light scattering (DLS) study for stability, poly dispersity, zeta potential and droplet size of nanoemulsions using Malvern Zetasizer. This work would be carried out in the DEI, Agra. Fourier transform Infrared Spectroscopic (FTIR) would be done to know the functional moieties and Transmission Electron Microscopic (TEM) analysis for evaluating exact shape and size of the droplets. FTIR and TEM characterization studies will be done at AIRF, JNU, New Delhi and AIIMS, New Delhi respectively.

Collection technique of experimental animal: For this, the protocol will be approved through institutional ethical committee. Primary level schools will be selected in the rural settings of Agra for this study. Permission would be acquired from the school Principal and also informed consent would be obtained from parents. Heavily infested children will be selected, and asked to come to the school after washing the hair with only water. Samples will be collected with the help of fine-toothed comb. New

comb will be used for each child and after collection target animal will be carefully transferred to the paper bags and transported to the laboratory for screening and identification.

A questionnaire may be framed for acquiring information about the usage of shampoos, soaps or other stuff used to wash hair by the children with the help of their parents.

Bioassay for pediculocidal activity of nanoformulations: A filter-paper contact lethal-time bioassay will be done. Lice will be exposed to various concentrations of formulated material to filter papers. Negative Control filter papers will be impregnated by only water or 1% surfactant for positive control permethrin will be use. After drying for 2 min, each filter paper will be placed on the bottom of a petridish. Groups of 10 females will be placed on each petridish containing a few

strands of human hair and covered with a lid. Treated and control females and eggs will be held at the same conditions used for colony maintenance. Adult mortalities will be determined every 5 min for 3 h. Observations will be taken under dissecting microscope and absence of any movement would be considered as mortality. All treatments will be in replicated thrice. The median lethal time (LT_{50}) values will be calculated (Oladimeji *et al*. 2000).

Statistical analysis: Data will be calculated using Graph pad prism statistical software. Various groups will be compared and test of significance will be calculated to see the difference in bioactivity of variousformulations.

The information generated through the extensive survey work and validation of herbal nanoformulation aspediculocide would be utilized in creating awareness campaigning in the schools children with the help of school administration.

PILOT STUDY

- **Preparation of nanoemulsion from one oil and optimization of fabrication protocol**

We have prepared the nanoformulation of different ratios of oil in water nanoemulsion by sonication. Firstly, oil (neem) and water along with surfactant and camphor are mixed in appropriate ratios by continuous stirring then lead to sonication and the protocol was optimized in the laboratory.

- **Preliminary study on the bioefficacy of prepared nanoemulsion with promisingpediculocidal activity.**

We have also conducted the pilot study on the efficacy of nanoemulsions of neem oil and observed that at 6000 ppm after 8 hour 100% mortality was observed for neem oil and there was an increase in the efficacy for the camphor based neem oil nanoemulsion as 100% mortality was obserbed at lowe concentration after 3 to 4 hour of exposure. Now, we will be planning to go withthe narrower range and the most stable nanoemulsion with their characterizations.

PLAN OF WORK

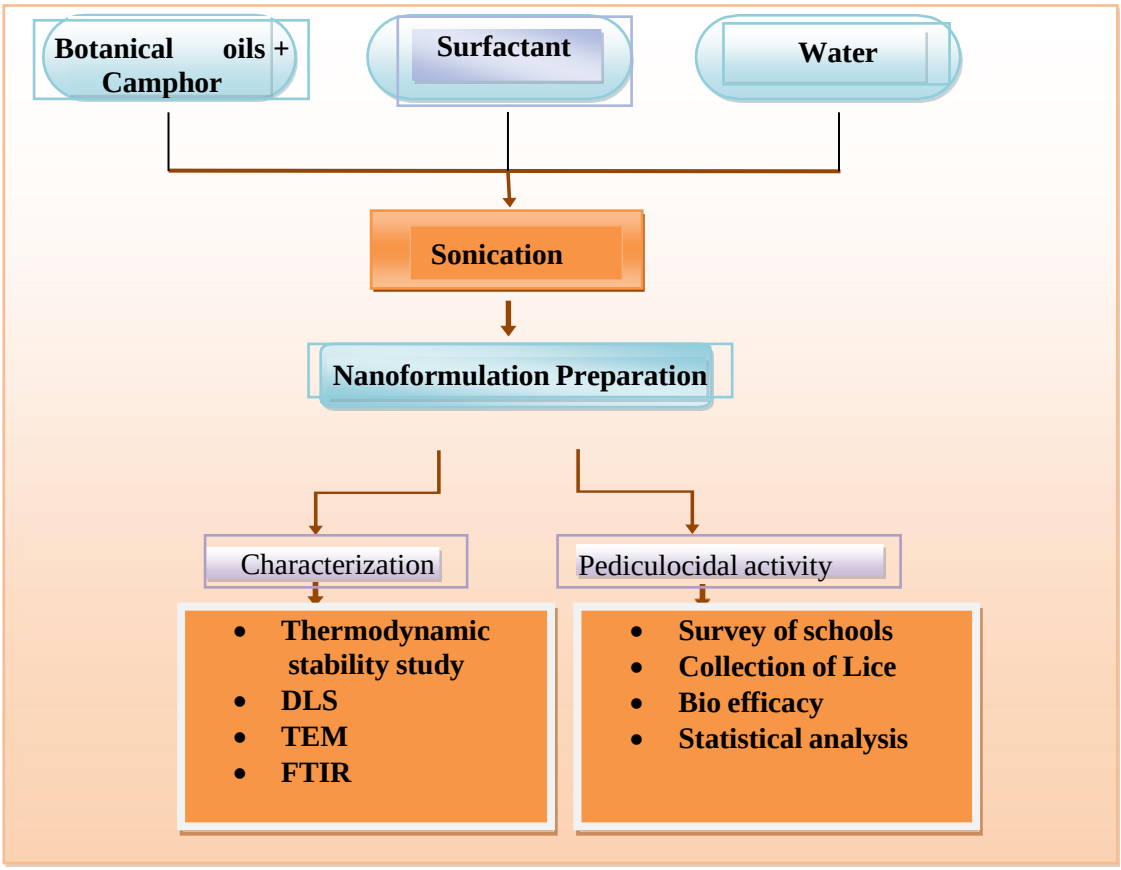

Figure 6 Plan of work

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